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Efficacy of Daptomycin in Implant-Associated Infection Due to Methicillin-Resistant *Staphylococcus aureus*: Importance of Combination with Rifampin[▽]

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Limited treatment options are available for implant-associated infections caused by methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA). We compared the activity of daptomycin (alone and with rifampin [rifampicin]) with the activities of other antimicrobial regimens against MRSA ATCC 43300 in the guinea pig foreign-body infection model. The daptomycin MIC and the minimum bactericidal concentration in logarithmic phase and stationary growth phase of MRSA were 0.625, 0.625, and 20 μg/ml, respectively. In time-kill studies, daptomycin showed rapid and concentration-dependent killing of MRSA in stationary growth phase. At concentrations above 20 μg/ml, daptomycin reduced the counts by >3 $\log_{10}$ CFU/ml in 2 to 4 h. In sterile cage fluid, daptomycin peak concentrations of 23.1, 46.3, and 53.7 μg/ml were reached 4 to 6 h after the administration of single intraperitoneal doses of 20, 30, and 40 mg/kg of body weight, respectively. In treatment studies, daptomycin alone reduced the planktonic MRSA counts by 0.3 $\log_{10}$ CFU/ml, whereas in combination with rifampin, a reduction in the counts of >6 $\log_{10}$ CFU/ml was observed. Vancomycin and daptomycin (at both doses) were unable to cure any cage-associated infection when they were given as monotherapy, whereas rifampin alone cured the infections in 33% of the cages. In combination with rifampin, daptomycin showed cure rates of 25% (at 20 mg/kg) and 67% (at 30 mg/kg), vancomycin showed a cure rate of 8%, linezolid showed a cure rate of 0%, and levofloxacin showed a cure rate of 58%. In addition, daptomycin at a high dose (30 mg/kg) completely prevented the emergence of rifampin resistance in planktonic and adherent MRSA cells. Daptomycin at a high dose, corresponding to 6 mg/kg in humans, in combination with rifampin showed the highest activity against planktonic and adherent MRSA. Daptomycin plus rifampin is a promising treatment option for implant-associated MRSA infections.

Implants are increasingly used in modern medicine to replace a compromised biological function or missing anatomical structure. Periprosthetic infections represent a devastating complication, causing high rates of morbidity and consuming considerable health care resources. Implant-associated infections are caused by microorganisms growing adherent to the device surface and embedded in an extracellular polymeric matrix, a complex three-dimensional structure called a microbial biofilm (8). Bacterial communities in biofilms cause persistent infection due to increased resistance to antibiotics and the immune system and the difficulty with eradicating them from the implant (6).

Staphylococcus aureus is one of the leading pathogens causing implant-associated infections. Successful treatment requires the use of bactericidal drugs acting on surface-adhering microorganisms, which predominantly exist in the stationary growth phase. Previous in vitro, experimental, and clinical studies demonstrated that rifampin (rifampicin)-containing antimicrobial regimens were able to eradicate staphylococcal biofilms and cure implant-associated infections (23, 25). Quinolones are often used in combination with rifampin in order to prevent the emergence of rifampin resistance (4, 19, 21). However, methicillin (meticillin)-resistant *S. aureus* (MRSA) strains are often resistant to quinolones. In addition, MRSA strains were recently shown to have decreased susceptibility to vancomycin, reducing the efficacy of this drug. Therefore, alternative drugs for use in combination with rifampin against implant-associated infections are needed (12, 20).

Daptomycin is a negatively charged cyclic lipopeptide with bactericidal activity against gram-positive organisms, including MRSA (17). The drug inserts into the bacterial cytoplasmic membrane in a calcium-dependent fashion, leading to rapid cell death without lysis, and causing only minimal inflammation (15). Daptomycin has been well tolerated in healthy volunteers dosed with up to 12 mg/kg of body weight intravenously for 14 days (2). Only limited data on the use of daptomycin in combination with rifampin against staphylococcal implant-associated infections are available.

In this study, we investigated the activity of daptomycin against MRSA ATCC 43300 in vitro. In addition, we evaluated

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the activity of daptomycin in combination with rifampin in a cage-associated infection model in guinea pigs and compared the efficacy of the treatment with the efficacies of three other antibiotics commonly used against MRSA, vancomycin, linezolid, and levofloxacin (alone and in combination with rifampin).

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MATERIALS AND METHODS

Study microorganisms. *S. aureus* strain ATCC 43300, which is resistant to methicillin and which is susceptible to rifampin, vancomycin, linezolid, and levofloxacin, was studied. For the testing of rifampin resistance, rifampin-resistant clinical *S. aureus* strain T4050 and rifampin-susceptible laboratory *S. aureus* strain ATCC 29213 were used. The strains were stored at −70°C by using the cryovial bead preservation system (Microbank; Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada). For preparation of the inoculum, single beads were transferred to 1 ml of sterile trypticase soy broth (TSB; Becton Dickinson and Company, Le Pont de Claix, France) and incubated for 7 h at 37°C. This preculture was then diluted 1:100 in fresh TSB and incubated overnight at 37°C without shaking. The bacteria were washed twice and resuspended in sterile and pyrogen-free 0.9% saline to the desired concentration. Bacterial concentrations were determined by plating of aliquots from appropriate dilutions on agar, followed by colony counting after 24 h of incubation at 37°C.

Antimicrobial agents. Daptomycin for injection was supplied by Novartis Pharma Schweiz AG (Bern, Switzerland). A stock solution of 50 mg/ml was prepared in sterile and pyrogen-free 0.9% saline. All other solutions were prepared in sterile water. Rifampin (Sandoz AG, Steinhausen, Switzerland) was prepared as a 60-mg/ml stock solution. Levofloxacin hemihydrate injectable solution (5 mg/ml) was purchased from Aventis Pharma AG (Zurich, Switzerland). Vancomycin was supplied by Teva Pharma AG (Aesch, Switzerland), and a stock solution of 50 mg/ml was prepared. Linezolid was provided as a purified powder from the manufacturer (Pfizer AG, Zurich, Switzerland), and a stock solution of 2.5 mg/ml was prepared.

In vitro antimicrobial susceptibility. A standard inoculum of 1×10^5 to 5×10^5 CFU/ml of MRSA strain ATCC 43300 was used. The MIC and the minimal bactericidal concentration (MBC) in the logarithmic growth phase (MBC_{log}) were determined by using twofold dilutions of antimicrobial agents in Mueller-Hinton broth supplemented with 50 mg/liter calcium ions ($CaCl_2$), according to the CLSI (formerly the NCCLS) guidelines (3). This concentration of calcium is necessary for the antimicrobial activity of daptomycin to be exhibited (1). The MIC was the lowest drug concentration that inhibited visible bacterial growth. The MBC_{log} was defined as the lowest antimicrobial concentration which killed ≥99.9% of the initial bacterial count (i.e., ≥3 $\log_{10}$ CFU/ml) in 24 h (10). In addition, the MBC was determined also in the stationary (nongrowing) growth phase (MBC_{stat}), reflecting the characteristics of microorganisms causing implant-associated infections. MBC_{stat} was determined by using overnight cultures of *S. aureus* in nutrient-limited medium (0.01 M phosphate buffered saline [PBS], pH 7.4) containing 0.1% glucose and 50 mg/liter calcium ions. In this medium, the bacterial counts remained stable for up to 48 h. MBC_{stat} was defined as the lowest concentration which reduced the inoculum by ≥99.9% in 24 h. The experiments were performed in triplicate.

Time-kill study in stationary growth phase. Glass tubes containing 10 ml PBS supplemented with 50 mg/liter calcium ions and 0.1% glucose were incubated with daptomycin at concentrations representing 4×, 8×, 16×, 32×, 64×, and 128× the MIC of the test strain at 37°C without shaking. Bacterial survival in the antimicrobial-free culture served as a control. To determine whether the inoculum size affects the killing activity of daptomycin, a low initial inoculum (3×10^5 CFU/ml) and a high initial inoculum (5×10^6 CFU/ml) were tested. For the high-inoculum assays, PBS with 50 mg/liter calcium ions was supplemented with 0.001% TSB to keep the bacterial counts in the antimicrobial-free culture stable for at least 24 h. Colony counts were determined immediately before addition of daptomycin (0 h) and after 2, 4, 6, 8, and 24 h of incubation with daptomycin at the appropriate concentrations. Before sampling of the probes, the tubes were gently vortexed and colony counts were determined by plating aliquots of appropriate dilutions on Mueller-Hinton agar. A bactericidal effect was defined as a ≥3-$\log_{10}$ (≥99.9%) reduction of the initial bacterial count (11). The experiments were performed in triplicate.

Animal model. We used a guinea pig model of foreign-body infection which was established by Zimmerli et al. (24). Guinea pigs (Charles River, Sulzfeld, Germany) were kept in the Animal House of the Department of Biomedicine, University Hospital Basel. The animal experiments were performed according to the regulations of Swiss veterinary law. In brief, four sterile polytetrafluoroethylene (Teflon) tubes (10 by 30 mm) perforated with 130 holes (Angst + Pfister AG, Zurich, Switzerland) were aseptically implanted into the flanks of male guinea pigs weighing at least 500 g. The animals were anesthetized with an intramuscular injection of ketamine (20 mg/kg; Parke-Davis, Zurich, Switzerland) and xylazine (4 mg/kg; Gräub, Bern, Switzerland). The experiments were started after complete wound healing (i.e., approximately 2 weeks after surgery). Before each experiment, the cages were checked for sterility by culturing the aspirated cage fluid. The guinea pigs were weighed daily to monitor their well-being during the experiment and to adjust the antibiotic doses.

Pharmacokinetic study. Pharmacokinetic studies were performed with sterile tissue cages. A single dose of 20, 30, and 40 mg/kg daptomycin was injected intraperitoneally (three animals and 12 cages per dose group). Cage fluid was aspirated by percutaneous cage puncture at 1, 2, 4, 6, 8, 10, 12, and 24 h after drug administration. For each drug dose, fluid from six cages per time point (two cages per time point and animal) was collected. Aliquots of 150 μl of cage fluid were transferred to tubes containing 15 μl of filter-sterilized 5% polyanetholsulfonic acid sodium salt (Sigma-Aldrich, Buchs, Switzerland), mixed by hand, and centrifuged at $2{,}100 \times g$ for 7 min. The supernatant was stored at −20°C until further analysis.

(i) High-performance liquid chromatography assay, followed by mass spectrometry. Daptomycin standards were prepared in cage fluid by spiking cage fluid from untreated animals with daptomycin solution in water-methanol (1/1) to give concentrations in the range of 0.2 to 150 μg/ml. Two hundred microliters of precipitation solution (methanol, acetonitrile, 1 mM zinc sulfate) containing 2 μg of CB183253 (internal standard) was added to 50 μl of each of the standards, samples, and controls. After vortexing of the samples and centrifugation, 100 μl of the supernatant was diluted with water-methanol (1/1) and 10 μl was injected into the liquid chromatography-mass spectrometry apparatus (TSQ; Thermo Fisher Scientific). Separation of the components was performed on a C_{18} column (Uptisphere; particle size, 5 μm; 125 by 2 mm) by using acetonitrile and 0.1% formic acid as the mobile phase. Daptomycin was quantified by analyzing m/z 811 → 341, and the internal standard was quantified by analyzing m/z 837 → 393. The daptomycin concentrations were calculated by linear regression of the peak ratios between daptomycin and the internal standard.

(ii) Pharmacokinetic parameters. Individual concentration-time data were analyzed by using the WinNonlin software package (Pharsight Corp., Mountain View, CA). For each time point, the mean fluid concentration of the six cages was used. Mean ± standard deviation (SD) values of the peak (maximum) concentration (C_{max}), the time required to reach C_{max} (T_{max}), the trough (minimum) concentration at 24 h after dosing (C_{min}), the half-life ($t_{1/2}$), and the area under the concentration-time curve (AUC) from time zero to 24 h (AUC_{0-24}) were calculated.

Antimicrobial treatment study. Cages were infected with the MRSA test strain by percutaneous injection of 200 μl bacterial suspension containing 4×10^6 CFU (day 0). The establishment of an infection was confirmed by quantitative culture of cage fluid 3 days later, immediately before the start of treatment. Three animals were randomized into each of the following 10 treatment groups: saline (control), rifampin at 12.5 mg/kg alone, linezolid at 50 mg/kg plus rifampin at 12.5 mg/kg, levofloxacin at 10 mg/kg plus rifampin at 12.5 mg/kg, vancomycin at 15 mg/kg alone and in combination with rifampin at 12.5 mg/kg, and daptomycin at 20 mg/kg and 30 mg/kg alone and in combination with rifampin at 12.5 mg/kg. The antimicrobial agents were given intraperitoneally for 4 days. The dosing interval was 12 h for all drugs except daptomycin, which was given every 24 h.

(i) Efficacy of treatment against planktonic bacteria. Bacterial counts (median and interquartile range) were determined before the start of treatment (i.e., day 3), during treatment (i.e., day 5), and 5 days after the completion of treatment (i.e., day 12). The efficacy of each treatment against planktonic bacteria in cage fluid was expressed as the difference in the bacterial counts ($\Delta\log_{10}$ CFU/ml) before and 5 days after the completion of treatment and the clearance rate (in percent), defined as the number of cage fluid samples without growth of MRSA divided by the total number of cages in the individual treatment group.

(ii) Efficacy of treatment against adherent bacteria. Five days after the end of treatment (i.e., day 12), the animals were sacrificed and the tissue cages were removed under aseptic conditions and incubated at 37°C in 5 ml TSB. After 48 h of incubation, 100 μl of the cage culture was spread on Columbia sheep blood agar plates (Becton Dickinson) and analyzed for bacterial growth. A positive culture of MRSA was defined as a treatment failure. The efficacy of treatment against adherent bacteria was expressed as the cure rate (in percent), defined as

TABLE 1. In vitro susceptibility of MRSA ATCC 43300

Antibiotic[a]	MIC (μg/ml)	MBC_{log} (μg/ml)	MBC_{stat} (μg/ml)	MBC_{stat}/MBC_{log} ratio
DAP	0.625	0.625	20	32
RIF	0.01	0.08	2.5	31
VAN	1	2	32	16
LZD	2.5	>20	>20	NA[b]
LVX	0.16	0.63	>20	>32

[a] DAP, daptomycin; RIF, rifampin; VAN, vancomycin; LZD, linezolid; LVX, levofloxacin.
[b] NA, not applicable.

the number of cages without growth divided by the total number of cages in the individual treatment group.

Emergence of antimicrobial resistance in vivo. Positive cultures of samples from explanted cages were screened for the in vivo emergence of resistance to rifampin, vancomycin, and daptomycin. In addition, all positive cultures of samples from cage fluid were screened for rifampin resistance. Colonies were collected from subcultures on agar; suspended in saline to the turbidity of a McFarland 0.5 standard; and spread on Mueller-Hinton agar plates containing 2 μg/ml of daptomycin, 1 μg/ml of rifampin, or 16 μg/ml of vancomycin. The plates were incubated at 37°C and screened for growth after 24 h.

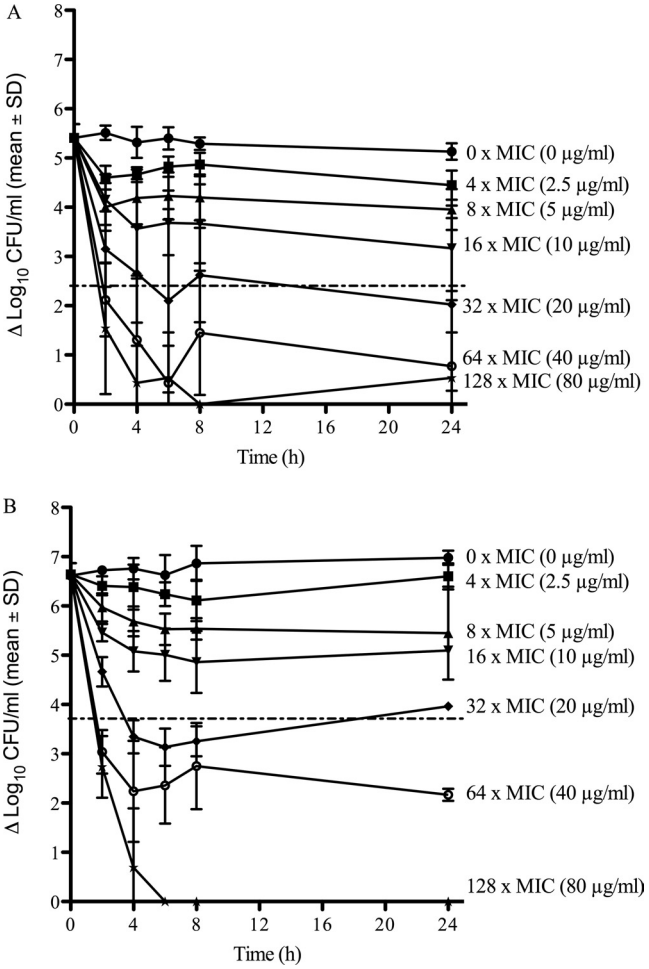

FIG. 1. Time-kill curve of a low inoculum (3×10^5 CFU/ml) (A) and a high inoculum (5×10^6 CFU/ml) (B) of MRSA in stationary growth phase exposed to increasing daptomycin concentrations (2.5 μg/ml to 80 μg/ml) corresponding to 4× to 128× MIC. Values are means ± SDs. The experiments were performed in triplicate. The horizontal dotted lines indicates a 3-log_{10} reduction of the numbers of CFU/ml from the initial inoculum.

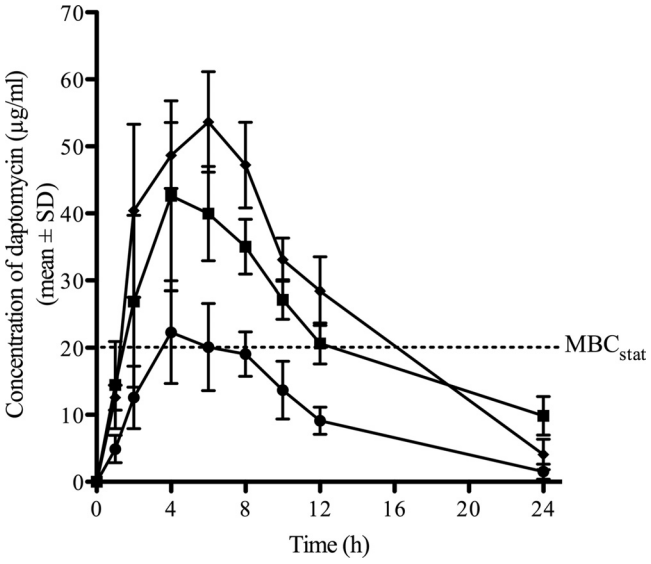

FIG. 2. Pharmacokinetics of daptomycin in sterile cage fluids after administration of single intraperitoneal doses of daptomycin at 20 mg/kg (circles), 30 mg/kg (squares), and 40 mg/kg (diamonds). Values are means ± SDs. The horizontal dotted line indicates the MBC_{stat} of MRSA for daptomycin.

Evaluation of antimicrobial toxicity. To evaluate the potential toxicity of daptomycin (20 mg/kg) administered with or without rifampin (three animals per group), histopathologic analysis of liver, kidney, and skeletal muscle tissues was performed. The corresponding organs of the saline-treated animals served as controls. The organs were fixed overnight in 4% buffered formalin, rinsed with PBS, and embedded into paraffin immediately after the animals were killed. Sections of 3 to 4 μm were mounted on slides and dried overnight at 37°C. The specimen sections were stained with hematoxylin-eosin and inspected by light microscopy.

Statistical calculations. Comparisons were performed by the Mann-Whitney U test for continuous variables and the two-sided χ^2 test or Fisher's exact test for categorical variables, as appropriate. For all tests, differences were considered significant when P values were <0.05. The graphs in the figures were plotted with Prism (version 5.0a) software (GraphPad Software, La Jolla, CA).

RESULTS

In vitro antimicrobial susceptibility. Table 1 summarizes the in vitro susceptibility of MRSA ATCC 43300. Of the antibiotics tested, rifampin showed the lowest MBC_{stat} (2.5 μg/ml), followed by daptomycin (20 μg/ml) and vancomycin (32 μg/ml), whereas linezolid and levofloxacin did not kill MRSA in the stationary growth phase. The MBC_{stat} was at least 16-fold higher than the MBC_{log} for all agents (except linezolid, which had only a bacteriostatic effect).

In vitro time-kill study in stationary growth phase. In the low-inoculum and the high-inoculum studies, the bacterial counts remained within ±5% of the initial inoculum in the antimicrobial-free culture for 24 h. The time-kill curves in Fig. 1 demonstrate that daptomycin had rapid and concentration-dependent bactericidal activity against stationary-phase MRSA with a low inoculum (Fig. 1A) as well as a high inoculum (Fig. 1B). At 20 μg/ml (32× MIC), which corresponded to the MBC_{stat}, daptomycin reduced the counts by ≥3 log_{10}

TABLE 2. Pharmacokinetic parameters of daptomycin in cage fluid after a single intraperitoneal dose and linked to the in vitro susceptibility parameters of the MRSA strain tested[a]

Dose (mg/kg)	C_{max} (μg/ml)	C_{min} (μg/ml)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{0-24} (μg · h/ml)	C_{max}/MIC	C_{max}/MBC_{log}	C_{max}/MBC_{stat}	AUC > MBC_{stat}/AUC_{0-24}
20	23.1 ± 7.0	1.5 ± 1.1	6.0 ± 2.0	4.8 ± 1.7	247 ± 52	36.9 ± 11.2	36.9 ± 11.2	1.2 ± 0.3	0.05 ± 0.04
30	46.3 ± 8.8	9.8 ± 2.9	4.7 ± 1.2	8.7 ± 0.5	548 ± 65	74.0 ± 14.1	74.0 ± 14.1	2.3 ± 0.4	0.26 ± 0.04
40	53.7 ± 1.3	4.1 ± 2.3	6.0 ± 0.0	4.5 ± 1.2	662 ± 10	85.8 ± 2.1	85.8 ± 2.1	2.7 ± 0.1	0.39 ± 0.01

[a] Values represent means ± SDs.

CFU after 4 to 6 h. At concentrations above 20 μg/ml, daptomycin reduced the counts by >3 $\log_{10}$ CFU/ml in 2 to 4 h.

Pharmacokinetic study. Figure 2 shows the concentration-time curves in sterile cage fluid after the administration of a single intraperitoneal dose of 20, 30, or 40 mg/kg daptomycin. Table 2 summarizes the values of the pharmacokinetic parameters calculated. For all three doses administered, the peak C_{max}s were above the MBC_{stat}s, whereas the concentrations of daptomycin after 24 h (C_{min}) remained above the MIC and MBC_{log} but not above the MBC_{stat}. The AUC_{0-24} increased with the dose from 247 to 662 μg · h/ml. The ratio of the AUC > MBC_{stat} to AUC_{0-24} increased in a dose-dependent manner from 5% (at 20 mg/kg) to 26% (at 30 mg/kg) and 39% (at 40 mg/kg).

Antimicrobial treatment study. Three days after inoculation, the bacterial counts surpassed the initial inoculum two- to threefold in all infected animals (data not shown). The planktonic bacterial counts (median ± interquartile range) in the cage fluid of the control group (treated with saline) increased by 1.4 ± 0.1 $\log_{10}$ CFU/ml (Fig. 3); no bacterial clearance (Fig. 4A) or spontaneous cure (Fig. 4B) was observed in the untreated group.

(i) Efficacy of treatment against planktonic bacteria. Figure 3 shows the killing of planktonic bacteria in cage fluid 5 days after the completion of therapy (compared to the bacterial counts before treatment start). By the use of monotherapy, the planktonic bacterial counts increased by <1 $\log_{10}$ CFU/ml with vancomycin or daptomycin at 20 mg/kg and decreased by 0.3 $\log_{10}$ CFU/ml with daptomycin at 30 mg/kg. In combination

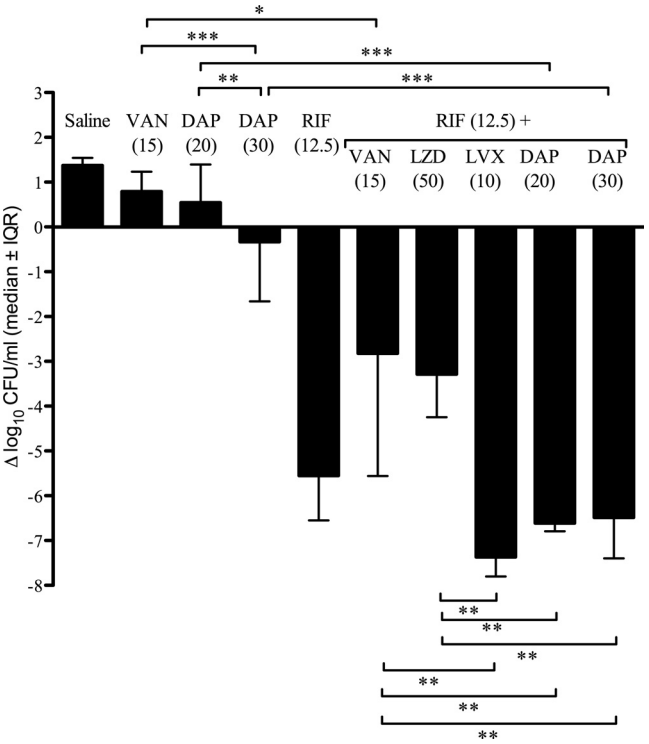

FIG. 3. Killing of planktonic MRSA in cage fluid 5 days after the completion of therapy. Positive values on the *y* axis denote the net growth and negative values denote the net killing. Values are medians ± interquartile ranges. The numbers in parentheses indicate the dose (in mg/kg) administered twice daily for all drugs except daptomycin, which was administered once daily. DAP, daptomycin; RIF, rifampin; VAN, vancomycin; LZD, linezolid; LVX, levofloxacin; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

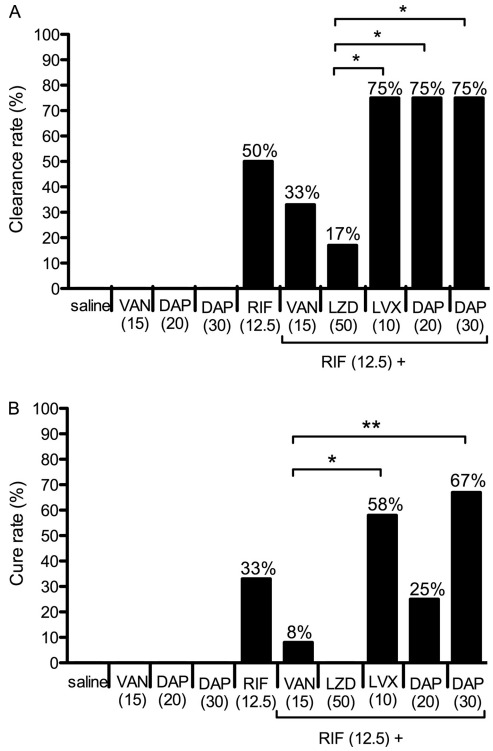

FIG. 4. Clearance rate of planktonic MRSA (A) and cure rate of adherent MRSA in explanted cages (B). The numbers in parentheses indicate the dose (in mg/kg) administered twice daily for all drugs except daptomycin, which was administered once daily. DAP, daptomycin; RIF, rifampin; VAN, vancomycin; LZD, linezolid; LVX, levofloxacin; *, $P < 0.05$; **, $P < 0.01$.

TABLE 3. Rates of emergence of rifampin resistance in cage fluid during and after treatment (planktonic bacteria) and in culture from explanted cages (adherent bacteria)

Treatment (dose)[a]	Planktonic bacteria[b]		Adherent bacteria[c] after treatment (day 12)
	During treatment (day 6)	After treatment (day 12)	
RIF (12.5)	2/12 (17)	2/12 (17)	3/12 (25)
VAN (15) + RIF (12.5)	4/12 (33)	5/12 (42)	7/12 (58)
LZD (50) + RIF (12.5)	0/12 (0)	0/12 (0)	1/12 (8)
LVX (10) + RIF (12.5)	0/12 (0)	0/12 (0)	0/12 (0)
DAP (20) + RIF (12.5)	0/12 (0)	0/12 (0)	2/12 (17)
DAP (30) + RIF (12.5)	0/12 (0)	0/12 (0)	0/12 (0)

[a] The doses are in mg/kg and were administered every 12 h for all drugs except daptomycin, which was administered every 24 h. RIF, rifampin; VAN, vancomycin; LZD, linezolid; LVX, levofloxacin; DAP, daptomycin.
[b] The data represent the number of cage fluid specimens with rifampin-resistant colonies/total number of all cage fluids (percent).
[c] The data represent the number of cage cultures with rifampin-resistant colonies/total number of cage cultures (percent).

with rifampin, levofloxacin and daptomycin at 20 and 30 mg/kg killed planktonic MRSA more efficiently (7.4 $\log_{10}$, 6.6 $\log_{10}$, and 6.5 $\log_{10}$ CFU/ml, respectively) than linezolid or vancomycin (3.3 $\log_{10}$ and 2.8 $\log_{10}$ CFU/ml, respectively) ($P < 0.01$ for all groups). In comparison to monotherapy, vancomycin plus rifampin was significantly more active against planktonic bacteria ($P = 0.019$). Similarly, daptomycin performed significantly better in combination with rifampin ($P < 0.0001$) in a manner that was independent of the dose administered.

Figure 4A shows the rate of clearance of planktonic bacteria in cage fluid. Vancomycin and daptomycin monotherapy were unable to clear planktonic MRSA. In combination with rifampin, levofloxacin and daptomycin showed higher clearance rates (all 75%) than linezolid (17%), vancomycin (33%), and rifampin (50%) alone.

(ii) Efficacy of treatment against adherent bacteria. Figure 4B shows the efficacy of treatment against adherent bacteria. Vancomycin and daptomycin (at both doses) were unable to cure any cage-associated infection when they were given as monotherapy, whereas rifampin alone cured the infections in 33% of the cages. In combination with rifampin, levofloxacin (58%) and daptomycin at 30 mg/kg (67%) cured significantly more infected cages than vancomycin (8%) and linezolid (0%).

Emergence of antimicrobial resistance in vivo. Table 3 shows the rates of emergence of rifampin resistance in planktonic MRSA during and after rifampin monotherapy (both 17%) as well as in adherent MRSA after treatment (25%). Rifampin resistance emerged more often during therapy with vancomycin plus rifampin (58%) than during therapy with linezolid plus rifampin (8%) or daptomycin at 20 mg/kg plus rifampin (17%). Levofloxacin plus rifampin and daptomycin at 30 mg/kg plus rifampin completely prevented the emergence of rifampin resistance in planktonic as well as adherent bacteria. No MRSA strain in cage fluid cultures from animals treated with daptomycin or vancomycin alone or in combination with rifampin developed resistance to daptomycin or vancomycin (data not shown).

Evaluation of antimicrobial toxicity. In animals treated with daptomycin (20 mg/kg), no acute lesions in the kidneys, liver, or skeletal muscles, such as acute muscle fiber necrosis (rhabdomyolysis), were observed. In animals treated with daptomycin and rifampin, liver histology showed mild inflammation.

DISCUSSION

Daptomycin was highly bactericidal in the logarithmic growth phase as well as in the stationary growth phase of MRSA ATCC 43300. These in vitro studies suggested that daptomycin may be efficacious in eradicating MRSA implant-associated infections. We used the cage-associated infection model in guinea pigs, which has been validated for use for the evaluation of drug activity against implant-associated infections (7, 9, 25). In contrast to the cage model in mice and rats (14), no spontaneous cure of infected cages occurs in guinea pigs, which resembles the situation in humans. Assuming an approximately 50% penetration into cage fluid, daptomycin doses of 20, 30, and 40 mg/kg in guinea pig correspond to human doses of 4, 6, and 8 mg/kg, respectively (2, 5, 22). Therefore, daptomycin was used at 20 and 30 mg/kg in subsequent treatment studies with guinea pigs.

In the treatment studies, none of the monotherapy regimens tested (except rifampin monotherapy) cleared planktonic MRSA or eradicated adherent MRSA from the cages. It might be possible that the concentrations of daptomycin administered were not sufficiently high to eradicate biofilm-associated MRSA. In a recent study, daptomycin at a concentration of 64 μg/ml had improved activity against staphylococci embedded in a biofilm (16). Therefore, a higher concentration of daptomycin corresponding to human doses above 6 mg/kg should be examined in future studies with animals.

In contrast, when levofloxacin or daptomycin at a high dose (30 mg/kg) were combined with rifampin, they showed high degrees of efficacy against the adherent bacteria. These data suggest that addition of rifampin to quinolones or lipopetides is important for the eradication of staphylococcal implant-associated infections. Interestingly, in combination with rifampin, vancomycin and linezolid, both first-line drugs used against MRSA, had lower cure rates. Furthermore, a higher daptomycin dose (30 mg/kg versus 20 mg/kg) in combination with rifampin was associated with a higher cure rate. The importance of rifampin-containing regimens was also demonstrated in vitro, when rifampin in combination with daptomycin was significantly more effective in eliminating MRSA from the biofilm than daptomycin alone (13).

In a previous study (18), levofloxacin alone was unable to eradicate methicillin-susceptible *S. aureus*, even though quinolone monotherapy cured about half of the staphylococcal implant-associated infections in the clinical setting (25). This reflects the stringent experimental conditions which were applied in the present experiments, in which a high infecting inoculum, a lack of debridement of the infected cages, and a short duration of antibiotic treatment (4 days) were used. These conditions were chosen in order to better discriminate the differences in efficacies of the antibiotics tested and to determine the risk of emergence of rifampin resistance. Antimicrobial regimens effective in the present animal model will probably also be effective in the clinical setting.

Rifampin resistance emerged in adherent MRSA from cage cultures with rifampin monotherapy; the rate of resistance was higher with addition of vancomycin and lower with addition of

daptomycin at 20 mg/kg or linezolid. Addition of levofloxacin and daptomycin at a high dose completely prevented the emergence of rifampin resistance. These data show the importance of combining rifampin with an effective antibiofilm drug administered at a sufficient dose.

In conclusion, daptomycin at a high once-daily dose, corresponding to 6 mg/kg in humans, in combination with rifampin showed the highest activity against planktonic and adherent MRSA and prevented the emergence of rifampin resistance. The cure rate achieved with this combination was comparable to that achieved with levofloxacin plus rifampin but higher than the one with vancomycin plus rifampin, which could not prevent emergence of rifampin resistance. This raises concern about vancomycin combination therapy. Since health care-associated MRSA strains are increasingly resistant to quinolones, daptomycin in combination with rifampin presents a promising treatment option for implant-associated staphylococcal infections.

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